CHARACTERIZATION OF HEPATITIS B AMONG INCIDENTALLY DETECTED ASYMPTOMATIC HEPATITIS B SURFACE ANTIGEN (HBSAG) POSITIVE SUBJECTS IN EGYPT

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ABSTRACT

Background : Although chronic hepatitis B virus infections are common in Egypt, the incidentally discovered asymptomatic forms are very frequent. Their serological profile and clinical significance have not been determined.

Aim : To characterize the clinical, serological and histological liver damage among incidentally detected asymptomatic hepatitis B surface antigen (HBsAg)-positive subjects (IDAHS) in Egypt.

Methods : We prospectively studied 70 consecutive IDAHS patients who were long term HBsAg carriers. Tests for liver function, serological markers for HBV, HCV, HDV and schistosomiasis were done for all patients. HBV DNA was determined by the branched DNA technique and PCR at the core promotor/precore region and the S region. Liver biopsy specimens from 44 patients were studied and scored for activity and fibrosis stage by modified Knodell score and the METAVIR score. HBsAg and HBcAg were immunohistochemically evaluated in the liver tissue.

Results : Of the studied 70 patients, 57 (81.6%) were HBeAg-negative and 13 (18.4%) were

HBeAg-positive. There was statistically significantly elevated hepatic transaminases in HBeAg-positive patients when compared to HBeAg-negative patients. HBV DNA was detected in only 3% of patients by the b-DNA technique and in 97% by the PCR techniques. Coinfection with HDV was found in 4.2% of patients. Pathological examination of liver tissue revealed mild activity in 21 (47.7%) of patients. Also, 21 patients (47.7%) revealed mild to moderate expansion of portal areas while 7 patients (15.9%) showed bridging fibrosis and no patient was cirrhotic.

Conclusion : Among IDAHS subjects, the majority are HBeAg negative without elevation of hepatic transaminases. They sould be considered patients since viremia is detected in almost all cases using PCR technique, and histopathological evidence of chronic hepatitis B virus infection and liver damage is noted in varying degrees.

INTRODUCTION

Hepatitis B virus (HBV) is one of the most common pathogens in the world. Annually up to 1 million die due to the consequences of this infection such as cirrhosis and hepato-cellular carcinoma [1]. Some authors found that 12% of Egyptian patients with chronic liver disease were positive for HBsAg.[2]

HBV genotype D is the most prevalent in Egypt[3] . It is associated with more severe disease and may predict the occurrence of HCC in young patients. Incidentally detected asymptomatic hepatitis B surface antigen (HBsAg)-positive subjects (IDAHS) infected with genotype D were found to have higher histological activity index (HAI) as compared to genotype A.[4]

Among important hepatitis associated risk factors reported were parenteral injection, dental manipulation and hospitalization. Blood transfusion seemed to have very minor role.[5] Chronic HB carrier rate was significantly higher among bilharzial cases (12.5%) than non-bilharzial individuals (6.2%).[6]

The course and outcome of chronic hepatitis B virus infection is quite variable. Milder forms are non-progressive or only slowly progressive and are usually accompanied by the loss of serum HBV DNA and sero conversion from hepatitis B e antigen to e antibody (anti-HBe).[7]

The prevalence of HbeAg-negative pattern is quite variable allover the world, being high in Middle East countries. In Iran it was reported to be 88.3% of HBV infections.(8) Lower prevalence has been reported in France and the US (22% and 54% respectively). (9,10) HBeAg is a marker of active HBV replication and infectivity. Precore mutants don't produce HBeAg and may be actively replicating in the absence of detectable HBeAg in the serum. Patients with precore mutants may still have anti HBe antibodies in their sera. There are many reports that this mutant causes a more severe disease and is less responsive to treatment.(11) El-Zayadi et al,(12) reported that HBeAg – negative chronic hepatitis B represents more than 80% of chronic hepatitis B in Egypt. Hepatitis B virus mutations within the precore region were described in many Mediterranean countries. (7)

There are various methods for detecting HBV DNA in the serum which could be grouped into PCR assays and non PCR assays .The non PCR assays include the hybridization and branched DNA (b-DNA) assays which can detect up to 10^6 and 10^5 particles /ml respectively. The PCR assays can detect as low as 1-10 particles /ml.(13)

This study is a trial to characterize HBV infection in incidentally detected asymptomatic hepatitis B surface antigen (HBsAg)-positive (IDAHS) among Egyptian subjects.

PATIENTS AND METHODS

Patients:-

The study included 70 patients presented at the outpatient clinic of the liver and Gastroenterology units of Mansoura, Tanta and Ain Shams University Hospitals from July 2001 to May 2002.

All patients were incidently detected asymptomatic hepatitis B surface antigen (HBsAg) positive subjects (IDAHS) for at least 6 months. They were diagnosed during blood donation or during blood analysis before traveling abroad for work as having hepatitis B.

All patients were subjected to thorough history taking with stress upon the possible routes of transmissions of HBV.

Patients were excluded if they had one of the following:-

1- Concomitant hepatitis C or any liver disease other than HBV,HDV, and schistosomiasis
2- Severe renal, hepatic or heart disease.
3- Malignancy.

Methods :-

1- Biochemical liver function tests including s.biliribun, s.ALT, s.AST, prothrombin time and albumin, and complete blood picture were done. Serological testing for HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc IgM and total anti-HBc), HCV and HDV markers (IgM and IgG) were determined by enzyme-linked immunosorbent assay (ELISA; Abbot laboratory, Chicago, IL).Serological test for schistosomiasis was done by using the indirect hemagglutination assay (IHA).(14)

2- Testing for HBV DNA by the branched DNA technique and polymerase chain reaction (PCR) from pre-s/s and pre-c/c regions were done for all patients. (15)
3- Abdominal ultrasound.
4- Percutanious liver biopsy was performed for 44 patients using Menghini needle (14 G, internal diameter 1.6mm).

Pathology and Immunohistochemistry procedure for HBsAg and HBcAg :

Pathological processing for liver biopsy specimens was as follows; 4μ sections were prepared from paraffin blocks and stained for Hematoxyline & Eosin stain, Masson trichrom, Reticulin, PAS and PAS-diastase stains. Fibrosis and necroinflammatory changes were assessed and scored synchronously by modified Knodell score (Ishak score[16]) and METAVIR score.(17 & 18) HBsAg and HBcAg immunohistochemistry were applied routinely to all liver biopsy specimens. Monoclonal antibodies to HBsAg and polyclonal antibody for HBcAg were applied (Zymed, South San Francisco, CA.).

Statistical analysis

Statistical analysis was done by using SPSS statistical package for social science programs version 10,1999 . The data were parametric by using Kolmogrov- Smirnov test. The qualitative data were presented in the form of number and percentage. Chi-square with Yatest correction was used. The quantitative data was presented in the form of mean, standard deviation and range. Student t test was used for comparison of

two groups. Pearson correlation coefficient was used to study correlation between variables. Significance was considered when p less than 0.05.

RESULTS

The demographic data for the 70 asymptomatic chronic HBV patients including the possible risk factors for transmission of injection are shown in table (1).

Biochemical and hematological results:-

Normal hepatic transaminases was found in 97 % of cases. Only 2 patients had elevated liver enzymes who were also HBeAg-positive and one of them had antibodies against HDV.

The liver function tests and hematological parameters are shown in table (2).

Serological markers :-

All studied 70 patients were positive for HbsAg, all were positive for anti –HBcIgG while anti-HBcIgM was negative in all patients.13 (18.4%) were positive for HbeAg while 57 (81.6%) were negative for HbeAg. Anti-HBs was identified in 7.1% of patients, also, anti-HDV IgG was seen in only three patients (4.3%). Only eleven patients (15.7%) were positive for schistosomiasis by the IHA test.

The differences in liver function tests and haematological profile between HBeAg-positive and HBeAg-negative patients are shown in table (4). There was significantly elevated ALT and AST levels in HBeAg-positive patients in comparison to HBeAg-negative patients ($P < 0.001$).

Molecular biological techniques for detection of HBV DNA :-

The use of bDNA technique detected HBV DNA in only 2.9 % of patients who were also HBeAg-positive. While, the use of PCR for detection of HBV DNA either by the pre-s/s or the pre-c/c technique was highly sensitive and were identical to each other. Using PCR based assays, HBV DNA was detected in most patients with anti-HBe (97.1%). (Table 5).

Pathology

Different pathological parameters are listed in table 6. Steatosis was identified in 11 out of the 44 biopsed patients (25%) with only one patient showing massive steatosis and most of them exhibit mild steatosis (fig. 1).

Capillarization of sinusoids was identified in 11 (27%) patients, that was mostly mild or moderate.

As regard *necroinflammatory injury,* 21 (47.7%) patients revealed mild activity, with score range (4-8), and 4 patients (10.1%) revealed moderate activity with score 9. On the other hand 19 (43.2%) of biopsed patients revealed no or minimal activity by Ishak score. By METAVIR score 27 patients (61.4%) revealed different grades of activity.

As regard *fibrosis* stage, 21 patients (47.7%) revealed mild to moderate expansion of portal areas while 7 patients (15.9%) showed bridging fibrosis. 16 (36%) of patients revealed no evidence of fibrosis and no patient was cirrhotic.

In comparing HBeAg positive and HBeAg negative patients, no statistical difference was identified between the two groups as regard the necroinflammatory injury or fibrosis stage, both by applying Ishak and METAVIR scoring systems (Table 7). There was 100% concordance between the two scoring systems as regard stage of fibrosis, however, there was no such concordance in evaluation of necroinflammatory activity. By METAVIR, there is higher estimation of degree of necroinflammation.

Considering the correlation between pathological parameters and laboratory findings, the only significant correlations were identified between ALT, and modified HAI, fibrosis stage by Ishak and with fibrosis stage by METAVIR (table 8). Moreover, there was significant correlation between ALT and each of the individual parameters of necoinflammation in liver biopsy; PMN, portal inflammation and focal parenchymal necrosis. Platelet number was inversely correlated with HAI, and fibrosis stage, however, short of statistical significance.

Ground glass hepatocytes were seen mostly in clusters, separating uninvolved hepatocytes, without acinar zone distribution. Less commonly they were seen singly scattered. Clustring of hepatocytes associates lack of significant necroinflammatory injury. Involved hepatocytes ranged from <5% ->75% of hepatocytes.

Immunohistochemical staining for HBsAg & HBcAg

HBcAg was positive in 12 (27.2%) biopsed cases. It was mainly cyto-

plasmic with less evident nuclear reaction (<10% of hepatocytes). Predominant nuclear reaction was identified in 5 cases. The latter pattern was associating necroinflammatory activity. 8 (21.1%) out of the 38 HbeAg-negative patients revealed positive tissue HbcAg by immunohistochemistry. Only 4 out of the 6 patients HBeAg- positive were positive for HBcAg in liver biopsy, while the other two patients (33.3%) were negative.

HBsAg, was positive in all 44 cases, cytoplasmic mostly in clusters of hepatocytes, and less commonly singly scattered. Membranous staining was uncommon. It was seen either alone or joined with the cytoplasmic pattern. It was seen associated with nuclear HBcAg reaction.

Only two patients out of the biopsed 44 individuals demonstrated bilharzial granuloma in the liver tissue. The 42 other biopsy specimens revealed no stigmata of bilharzial affection.

Table 1: The demographic data for the studied 70 chronic HBV patients

Demographic parameters	N = 70 patients
Male / female	66 / 4
Age (year) (mean $\pm$ SD)	31.47 $\pm$ 7.81
Previous operations n(%)	10 (14.3%)
Blood transfusion n(%)	0 (0.0%)
Dentist consultation n(%)	65 (92.9%)
Alcoholic intake n(%)	0 (0.0%)
Parenteral treatment of schistosomiasis n(%)	2 (2.9%)
Drug abuse n(%)	0 (0.0%)
Schistosomiasis n(%)	11 (15.7%)

Table 2: Liver function tests and hematological parameters of the studied 70 patients.

Parameters	Mean ± SD
Albumin level (gm%)	4.39±0.29
Bilirubin level (mg%)	0.816±0.18
ALT level U/L	32.06±20.94
AST level U/L	28.51±12.5
Hemoglobin level (gm%)	12.97±1.07
WBCs count x 10^3	6.177±1.10
Platelet count x 10^6	209.63±52.98

ALT, alanine aminotransferase. AST, aspartate aminotransferase. WBCs, white blood cells.

Table (3) : Positive serological markers for the studied group.

Serological markers	N=70 patients
HBsAg	70 (100%)
HBeAg	13 (18.4%)
Anti-HBe	57 (81.6%)
Anti-HBcIgG	70 (100%)
Anti-HBs	5 (7.1%)
Anti-HDV IgG	3 (4.2%)
IHA test for schistosomiasis	11 (15.7%)

Table (4): Liver function tests and haematological profile between HBeAg-positive and HBeAg-negative patients

Parameters	HBeAg-ve (n=57) mean ± SD	HBeAg+ve (n=13) mean ± SD	P
ALT	27.42±4.72	52.38±23.21	0.001
AST	25.43±4.5	39.85±15.16	0.001
Albumin	4.34±0.285	4.36±0.032	0.71
Bilirubin	0.805±1.189	0.862±0.126	0.31
WBCs	6.29±1.149	5.671±0.72	0.23
Platelets	208.7±52.12	213.64±58.69	0.76
Hemoglobin	13.02±1.06	12.75±1.13	0.411

Table (5): Detection of HBV DNA by molecular biological techniques:

Detection technique	No = 70 patients No (%)
HBV DNA detection by bDNA	2 (2.9%)
HBV DNA detection by PCR pre-s/s	68 (97.1%)
HBV DNA detection by PCR pre-c/c	68 (97.1%)

Table (6) : Pathological parameters of the biopsied 44 patients

Parameters	Number	Mean	Median	SD	Range
Modified HAI: 0-18	44	4.18	4.5	2.81	0-9
Fibrosis score: 0-6	44	1.34	1.0	1.33	0-5
Steatosis	11/44 25%	0.36	0.00	0.72	0-3
Capillarization	12/44 27%	0.41	0.00	0.76	0-3

HAI, histological activity index.

Table (7): Comparison between HbeAg positive and HbeAg negative patients as regard the necroinflammatory injury and fibrosis, both by applying Ishak and METAVIR scoring systems

Parameters	Total (n=44)	HBeAg-ve (n=38)	HBeAg+ve (n=6)	p-value
Ishak score:				
Modified HAI (0-18)				
0-3	19 (43.2%)	17 (44.7%)	2 (33.3)	0.520
4-8	21 (47.7%)	17 (44.7%)	4 (66.7%)	
9	4 (10.1%)	4 (10.6%)	0 (0%)	
Fibrosis score (0-6)				
0	16 (36.4%)	13 (34.2)	3 (50%)	0.719
1-2	21 (47.7%)	19 (50%)	2 (33.3%)	
3-5	7 (15.9%)	6 (15.8%)	1 (16.7%)	
METAVIR score				
Activity (A0-3)				
A0	17 (38.6%)	14 (36.8)	3 (50%)	0.229
A1	18 (41%)	17 (44.7)	1 (16.7%)	
A2	6 (13.6%)	4 (10.6%)	2 (33.3%)	
A3	3 (6.8%)	3 (8%)	0 (0%)	
Fibrosis (F0-4)				
F0	16 (36.4%)	13 (34.2)	3 (50%)	0.766
F1	21 (47.7%)	19 (50%)	2 (33.3%)	
E2	5 (11.4%)	4 (10.6%)	1 (16.7%)	
F3	2 (4.5%)	2 (5.2%)	0 (0%)	

Table 8: Correlation between Laboratory Finding and Pathology of biopsed 44 cases

Histo pathology	ALT		AST		S.Albumin		Platelets		HGB.	
Parameters	r	p	r	p	r	p	r	p	r	p
HAI	0.53	<0.001	0.116	0.45	0.008	0.96	0.277	0.076	0.115	0.45
Fibrosis	0.46	<0.001	0.145	0.38	0.13	0.37	0.247	0.106	0.025	0.87
PMN	0.389	0.009	0.078	0.615	0.12	0.43	0.26	0.088	0.97	0.53
Protal Inflam.	0.402	0.007	0.045	0.77	0.043	0.78	0.27	0.072	0.015	0.30
Focal necrosis	0.378	0.011	0.07	0.65	0.108	0.485	0.121	0.43	0.013	0.93
Confluent nec.	0.16	0.278	0.06	0.66	0.032	0.83	0.098	0.52	0.013	0.93
Steatosis	0.025	0.87	0.13	0.38	0.14	0.35	0.098	0.52	0.093	0.54
Capilarisation	0.369	0.014	0.06	0.69	0.15	0.322	0.097	0.53	0.150	0.33
METAVIR										
A	0.29	0.652	0.12	0.43	0.062	0.69	0.098	0.52	0.028	0.85
F	0.358	0.017	0.104	0.503	0.16	0.29	0.208	0.173	0.088	0.57

There is significant correlation between ALT and each of HAI, fibrosis, PMN, portal inflammation, focal necrosis and fibrosis stage by METAVIR.

Table 9: The relationship between HBeAg and anti-HBe to tissue immunohistochemical reaction for HBcAg.

Tissue HBcAg	Number	Serum HBeAg	
	44	Positive (6)	Negative (38)
Positive	12 (27.2%)	4 (66.7%)	8 (21.1%)
Negative	32(72.8%)	2 (33.3%)	30 (78.9%)

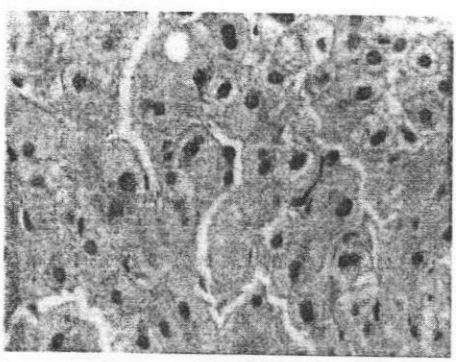

Fig. 1 : Hepatitis B; Ground glass cytoplasm (H&E X 400) .

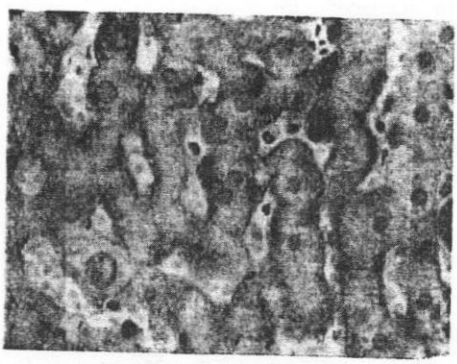

Fig. 2 : Positive membranous HBSAg. (Peroxidase X 400) .

DISCUSSION

This current study showed that 81.6% of our patients are HBeAg negative which is nearly identical to El-Zayadi et al, [12] who found that more than 80% of chronic hepatitis B cases were HBeAg negative . This high frequency HBeAg negative chronic HBV infection is comparable to other studies reported in Middle East and Mediterranean countries. [3, 8, 19, 20] However, this is different from the results reported from France (22%)[9] and the US (54%).[10] The frequency of HBeAg-positivity in our study was 18.4%, which is comparable to the data reported in subsharan Africa (20.5%)[21] and Iran (12%)[19] but lower than the 40% prevalence reported in the far east. (22) The geographic variation of e-CHB has a direct relation to the genotype distribution. Saudy et al.[3] had reported that HBeAg disappears early in patients with HBV genotype D, which is the predominant genotype in Egypt, because of early stop codon mutation.

The high prevalence of dentistry contact (93%) in our patients may direct our attention for further studies aiming at disclosure of the possible role of dentistry clinics in HBV transmission.

In our study, hepatic transaminases were normal in 97 % of the asymptomatic chronic HBV patients. All HBeAg-ve patients had normal liver enzymes, while two out of the 13 HBeAg positive patients revealed significantly elevated enzymes. The presence of HBeAg in the serum indicates active viral replication, higher infectivity and a potential for more liver damage. This is in agreement with Evants et al, [10] and Dienstage & Isselbacher. [23]

In the current study, only 3% of our patients were +ve HBV DNA in serum using b-DNA technique, however, using PCR pre-s/s or pre-c/c DNA was +ve in 97% which proves the higher sensitivity of detection by the latter technique. This in aggrement with Lindh et al, [24] and Kessler et al, [25] who found that using PCR assays, the majority of patients with chronic HBV infection, including those who are hepatitis B e antibody positive have detectable HBV DNA.

We found coexistence of HBsAg and anti-HBs in 7% of patients. The protective anti-HBs antibody is directed against the (a) determinant of HBsAg. In most instances of coincident HBsAg and anti-HBs, the anti-

bodies are directed against one of the determinants other than the (a) determinant and are unable to neutralize the circulating virions.[26] Coexistence of HBsAg and anti-HBs has been reported in 24% of HBsAg-positive individuals in Iran.[27]

In the present study 4.2% of asymptomatic chronic HBV infections have coexistent hepatitis delta virus antibodies. Positive anti-HD was reported in 8.3%[28] and in 10.5% [3] of otherwise healthy Egyptians positive for HBsAg.

Presence of early stages of fibrosis in 21 (47.7%) of the biopsed 44 IDAHS patients and even, presence of bridging fibrosis in 7 (15.9%) of them may reflect the importance of liver biopsy in assessment of such patients irrespective of serology or enzyme profile. Absence of cirrhosis in any of all biopsed 44 IDAHS patients, and even complete absence of fibrosis in 36% of the patients may reflect the relative favorable behavior of such individuals. Also, presence of significant necroinflammation (HAI 4-9) in >50% of biopsied patients is in contrary to Martinot-Peignoux et al,[29] and Ben Rejeb et al,[30] who reported minimal activity in such hepatitis B patients with normal enzymes. This in aggrement with Thakur et al,[4] who found that genotype D is associated with more severe liver disease

Presence of significant correlation between ALT, HAI and fibrosis may help in follow up of those patients. This is in aggrement with Yalcin et al [31] who reported that monitoring of ALT is of value in assessing hepatocellular damage in patients with chronic hepatitis B virus infection. They also, suggested that HBeAg-negative patients with elevated ALT levels and some with normal ALT levels should be considered highly infectious in the course of chronic HBV infection. Lack of significant difference between HBeAg positive and HBeAg negative patients groups as regard the necroinflammatory injury or fibrosis stage is in aggrement with Yalcin et al. [31]

In the present work, 21.1% of patients with negative serum HBeAg, demonstrated HBcAg in liver tissue by immunohistochemistry. On the other hand 33.3% of patients with positive serum HBeAg were found to be negative for HBcAg in liver tissue by immunohistochemistry. This may indicate that serum HBeAg status does not re-

flect the active replication of hepatitis B virus in liver tissue, and routine staining for HBsAg and HBcAg in chronic hepatitis B patients is valuable in reflecting hepatitis B pathology.

In agreement with Chu and Liaw, (32) membranous staining of HBsAg on the hepatocyte was seen associated with nuclear HBcAg reaction and thus can be recognized as a sensitive and specific marker of active hepatitis B virus replication. Predominant nuclear reaction of HbcAg pattern was associating necroinflammatory activity that reflect viral replication as it was reported by Chu et al.(33) This is in contrary with Sharma et al, (34) who found no significant correlation between the pattern of HBsAg or HBcAg expression and HAI score. The mechanism of intrahepatic shift of HBcAg from the nucleus to the cytoplasm and the decreased levels of viremia in this phase may be, at least in part, secondary to liver damage and regeneration. (35)

Steatosis in our patients was found in 11 patients (25%). It was mostly mild, comparable to that of Czaja et al (36) who identified fat deposition in 22% of chronic hepatitis B, however, lower than findings reported by Malhotra et al,(37) who found steatosis in 66.6% of chronic hepatitis B patients in comparison to 70% of HCV patients. On the other hand, shah et al,(38) found no steatosis in any of studied 34 chronic hepatitis B patients.

Conclusions :

We conclude that IDAHS subjects reveal positive HBV DNA with varying histopathological activity and fibrosis. Those are practically considered as patients, with predominance of HBeAg-negative pattern.

For complete diagnosis and proper selection for treatment; PCR for HBV DNA, together with routine immunohistochemical staining for HBsAg and HbcAg should be done and interpreted in the light of biochemical and serological results.

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